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SUBJECT OF INVESTIGATION

STUDIES ON THE MODE
OF
ACTION
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ANTIBACTERIAL DRUGS

RESPONSIBLE INVESTIGATOR

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"STUDIES ON THE MODE OF ACTION OF ANTIBACTERIAL DRUGS"

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A new antibiotic, mitomycin, has been isolated in our institute by Dr. Hata, from a new species of streptomycete, Streptomyces caespitosus. This substance was detected in the course of the screening program for the antitumor agents against the Ehrlich carcinoma. The antitumor activity was found to be in correlation with the antibacterial activity against B. subtilis PCI 219. The extraction of the active principle was carried out depending upon this antibacterial ctivity. As a result, the isolated principle was further separated into 3 active fractions. The names of mitomycin A, B and C were given to the respective fractions.
The mitomycins showed strong inhibitory activities against a variety of gram-positive and gram-negative bacteria in vitro, as shown in Table 1.
Among them, mitomycin A may be one of the most potential antibiotics which have so far been reported.

Table 1. Antibacterial Spectrum of Mitomycins

Test Organisms	Minimum Inhibitory Concentration A mcg	B mcg
Staphylococcus aureus 209-p	0.005	0.1
Diplococcus pneumoniae	0.01	0.1
Streptococcus hemolyticus	0.01	0.1
Corynebacterium diphtheriae	0.001	0.1
Escherichia coli	0.15	0.2
Klebsiella pneumoniae	0.15	0.005
Salmonella typhi	0.6	0.8
Shigella dysenteriae	0.15	0.8
Bacterium subtilis PCI 219	0.001	0.025

1. Inhibition of bacterial protein synthesis by mytomycin.

a. Materials and Methods.

(1) The organism. The organism used for this work was Staphylococcus aureus strain 209-p grown for 18 hrs. at 37°C on normal agar media and the cells were harvested and washed once with 0.1 M phosphate buffer pH 6.25 and resuspended the same buffer. The cell suspensions were subjected to freezing and thawing twenty times. The disintegrated material was centrifuged at 1,000 r.p.m. for 6 min.. This sediment was called Fraction I and the supernatant was resuspended 10 ml. of the buffer and recentrifuged at 6,000 r.p.m. for 30 min. and the sediment was refared to Fraction II, and the supernatant, Fraction III.

(2) Incubation mixtures. Incubation were carried out in 15 ml. centrifuge tubes containing the following: 0.1 ml. 0.06M Adenosin triphosphate; 0.4 ml. Hexose diphosphate solution containing the equivalent of 100 mg. barium salt/ml.; 0.5 ml. amino acid mixture A as discribed the previous paper, 0.5 ml. the solution of antibacterial drugs and 0.5 ml. bacterial cell fraction.

(3) Precipitation and estimation of protein-nitrogen. After incubating the tubes in a water bath at 37°C for 3 hrs, the same volume of 10% trichloracetic acid (ATC) was added to each tube and kept in ice box overnight. After centrifugation at 3,500 r.p.m. for 20 min., the sediments were washed once with 5% TCA. The nitrogen content of this precipitate was determined by digestion in microkjeldahal apparatus and colorimetric after nesslerization.

b. Results

Table 2. Inhibition of protein synthesis by Mitomycin

	Concentration mcg/ml	Inhibition %
Mitomycin C	50	0
	10	0
	5	0
	1	0
Chloramphenicol	50	100
	10	100
	5	78
	1	4
Leucomycin	50	97
	10	94
	5	94
	1	90

(1) In Table 2 are represented the degree of inhibition produced by a variety of inhibitors tested on the synthesis of protein. Inhibition is expressed as percentage rate of protein synthesis in the absence of added inhibitor. Mean value of controls without inhibitor is 60 mcg/mg dry wt. disrupted cell.

(2) Mitomycin C, although its high bacteriostatic activity, did not show any inhibitory power against the bacterial protein-synthesis.

(3) Chloramphenicol and Leucomycin showed high inhibitory activity against bacterial protein synthesis. The degree of inhibition of protein-synthesis was well correlated with their respective antibacterial activity.

2. Inhibition of beta-galactosidase induction by mitomycin.

a. Material and Methods.

(1) The organism. The organism employed for this experiment was also Staphylococcus aureus 209-p and three fractions of the disrupted cells were prepared quite same as the experiment 1.

(2) The drugs. Mitomycin, Chloramphenicol and Leucomycin were tested. Concentration of the antibacterial agents were adjusted to obtain a suitable level (100-0.05 mcg per ml. of final) in reactions mixture. The solution of drugs were stored in a refrigerator during the experiment and the activity of the compounds did not show any change.

(3) The enzyme induction and assay of its activity. The ability of cellular suspensions to synthesize beta-galactosidase was determined with the O-nitrophenyl-beta-d-galactoside method of Lederberg (1950) modified by Hurwitz et al. (1958). 0.2 ml. of each fractions contained in 15 ml. centrifuge tubes were added with 1 ml. of drug solution, 0.4 ml. of mixture B modified from medium A as follows: Na_2HPO_4 3.2%; KH_2PO_4 0.8%; Na-citrate 0.2%; $MgSO_4$ 0.04%; NH_4Cl 0.4%; Glucose 0.8% replaced with galactose as an inducer. The tubes were incubated at 37°C for 3 hrs. in a water bath. At the end of the incubation a few drop of toluol were added to make cryptic enzyme accessible to substrate. Then one ml. of M/200 O-nitrophenyl-beta-d-galactoside (ONPG) solution and 4 ml. of 0.2 M phosphate buffer pH 7.5 were added to the incubation mixture to measure enzyme activity and the tubes were reheated to 37°C and kept at this temperature for 15 min.. This reaction was stopped

after a given time interval by addition of 1 ml. of 1 M sodium Carbonate. After the centrifugation of the tubes to clarify the reaction mixture, the intensity of yellow colour developed in cell free fluid resulting from liberation of o-nitrophenyl by cleavage of the ONPG was estimated Pulfrich photometer with No. 53 filter.

h. **Result.**

Table 3. Inhibition of induced beta-galactosidase synthesis by Mitomycin

Mitomycin	Leucomycin	Chloramphenicol	Per cent inhibition
mcg/ml			%
5.0	-	-	100
1.0	-	-	100
0.5	-	-	60
0.1	-	-	11
0.05	-	-	0
	mcg/ml		%
-	6.3	-	100
-	3.2	-	100
-	1.6	-	69.2
-	0.8	-	38.4
-	0.4	-	5.1
-	0.2	-	0
-	0.1	-	0
-	0.05	-	0
		mcg/ml	%
-	-	6.3	100
-	-	3.2	100
-	-	1.6	83.8
-	-	0.8	50.2
-	-	0.4	16.2
-	-	0.2	10.8
-	-	0.1	9.0
-	-	0.05	0

(1) The result obtained were indicated in the Table 2. In this experiment the effects of graded concentration of the antibacterial drugs on beta-gala-ctosidase induction of Staphylococcus aureus were observed a range of 0.05 to 5.0 mcg/ml. Activity of beta-galactosidase is shown in per cent and the value of 100 per cent means the normal formation of the enzyme that is obtained in the absence of inhibitors. If the complete inhibition of the enzyme induction occurs, the value may reach to zero.

(2) Induction of beta-galactosidase of Fraction II, cell-free extract, was completely inhibited by mitomycin at the concentration 1.0 mcg/ml.. Fifty percent inhibition dose was 0.45 mcg/ml..

(3) Fifty percent inhibition dose of Chloramphenicol and alpha-Bromocinnamaldehyd is 0.1 mcg/ml. and 0.8 mcg/ml. respectivly.